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Involvement of Dopamine Receptor in the Actions of Non-Psychoactive Phytocannabinoids

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Abstract

Nematode *Caenorhabditis elegans (C. elegans)* exhibited a vigorous swimming behavior in liquid medium. Addition of dopamine inhibited the swimming behavior, causing paralysis in 65% of wild-type nematodes. Interestingly, phytocannabinoids cannabidiol (CBD) or cannabidivarin (CBDV), caused paralysis in 40% of the animals. Knockout of DOP-3, the dopamine D2-like receptor critical for locomotor behavior, eliminated the paralysis induced by dopamine, CBD, and CBDV. In contrast, both CBD and CBDV caused paralysis in animals lacking CAT-2, an enzyme necessary for dopamine synthesis. Co-administration of dopamine with either CBD or CBDV caused paralysis similar to that of either phytocannabinoid treatment alone. These data support the notion that CBD and CBDV act as functional partial agonists on dopamine D2-like receptors *in vivo*. The discovery that dopamine receptor is involved in the actions of phytocannabinoids moves a significant step toward our understanding of the mechanisms for medical uses of cannabis in the treatment of neurological and psychiatric disorders.

Keywords

Phytocannabinoid; Cannabidiol; Cannabidivarin; *C. elegans*

1. Introduction

The *Cannabis sativa*-derived non-psychoactive compound cannabidiol (CBD) and its propyl analog cannabidivarin (CBDV) are purported to have a broad range of therapeutic benefits stemming from anti-inflammatory, anti-nausea, anti-tumor, anti-convulsant, anxiolytic, and neuroprotective properties [1, 2] While these phytocannabinoids are known to act on a variety of molecular targets throughout the body and especially in the nervous system, our understanding of their mechanisms of action remains limited [1, 2].

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The neurotransmitter dopamine plays a central role in the regulation of learning, memory, emotion, cognition, and locomotion [3]. Numerous neurodegenerative and neuropsychiatric disorders have been associated with dopaminergic dysfunction [3]. Phytocannabinoids have been suggested as potential therapeutic agents for pathologies involving the dopamine system [4, 5]. However, there is limited information on the interactions between phytocannabinoids and dopamine receptors[6]. In this study, we investigated the ability of two non-psychotropic compounds, CBD and CBDV, to modulate dopaminergic signaling *in vivo*.

The nematode *Caenorhabditis elegans* shares a similar dopamine system with mammals. This provides a useful *in vivo* model for identifying pharmacological compounds able to target this key neuromodulatory system [7]. *C. elegans* possess a number of characteristics advantageous for pharmacological research, including a rapid life cycle, small size, ease of genetic manipulation and transparency, which facilitates visualization of cellular and developmental processes [8]. An estimated 60% of genes associated with human pathologies are present in *C. elegans*, whose genome was sequenced in 1998 [9–11]. A complete neuronal wiring diagram with extensively characterized synaptic connectivity has been constructed [12]. The molecular mechanisms underlying G protein-coupled receptor (GPCR) signaling in the nervous system are largely conserved between nematodes and humans. Key GPCRs of biogenic amines, including dopamine, present in nematodes are homologs of mammalian neurotransmitter receptors [13].

When immersed in a liquid environment, *C. elegans* exhibit vigorous swimming or ‘thrashing’ behavior that is regulated by endogenous dopamine and the DOP-3 (D2-like) receptor signaling pathway [14]. Swimming-based paralysis assays measuring locomotor behavior have been used extensively to study the functional roles of dopamine system, including receptors and transporters [14–19]. In this study, we used the *C. elegans* swimming assay to test the hypothesis that phytocannabinoids CBD and CBDV exert their actions *in vivo* by acting on the dopamine system.

2. Materials and Methods

2.1. Nematode strains and maintenance

The following *C. elegans* strains were obtained from the Caenorhabditis Genetics Center (University of Minnesota, Minneapolis, MN) and handled using standard conditions [20]: N2 Bristol wild-type, LX703 *dop-3(vs106)X*and CB1112 *cat-2(e1112)II.* nematodes were grown on *E. coli* OP50-seeded NGM agar plates at 15 °C for stock maintenance and 25 °C for behavioral assays.

2.2. Drugs

CBD and CBDV were purchased from Cayman Chemicals (Ann Arbor, MI) and dissolved in ethanol to generate 0.03, 0.3 and 3 mM stock solutions. Dopamine was purchased from Cayman Chemicals (Ann Arbor, MI) and dissolved in dH_2O to generate a 1 M stock solution.

2.3. Swimming assay

Behavioral assays were performed as previously described with some modifications [21]. Briefly, ten to fifteen late L4 hermaphrodite larvae were placed in a well containing 100μL K medium (2.36 g KCl, 3g NaCl in 1 L dH_2O) [22] and 1μL of either CBD, CBDV, or vehicle. The number of nematodes paralyzed versus swimming were recorded after 30 minutes. For each treatment group per genotype, six wells were scored per experiment and experiments were repeated on five separate days (n=30).

2.4. Statistics

All data was processed using GraphPad Prism (GraphPad Software, San Diego, CA). The ratios of paralyzed to non-paralyzed nematodes were compared between strains and drug treatments using one-way ANOVA, followed by Dunnett's post post-tests. Data are expressed as mean ± standard deviation and *p*-values of <0.05 were considered significant.

3. Results

3.1. Phytocannabinoid-induced paralysis of wild type nematodes

In the first set of experiments, a dose-response relationship analysis for two phytocannabinoids, CBD and CBDV, in N2 wild type nematodes swimming assay was performed (Figures 1A and 1B). No significant difference in swimming behavior was observed between K medium- and vehicle-treated nematodes. Dopamine, a known promoter of paralysis, was used as a positive control [23, 24]. As expected, wild type *C. elegans* showed an increase in paralysis when exposed to 50 mM dopamine, with 64.9% ± 6.5% of the nematodes paralyzed. Furthermore, both CBD and CBDV elicited a significant increase in paralysis at 30 μM concentration, compared to vehicle control, with 40.4% ± 9.4% and 39.8% ± 5.0% of animals paralyzed respectively (Figures 1A and 1B). Notably, their effect was about 60% in magnitude as compared to those produced by 50 mM dopamine treatment. Lower concentrations (0.3 μM and 3 μM) of the two phytocannabinoids failed to significantly induce paralysis.

3.2. Phytocannabinoid-induced paralysis in dop-3 mutants

Based on these results, the mechanism by which these cannabinoids promote paralysis was next examined. *C. elegans* express the DOP-3 receptor, a homologue of the mammalian D2-like receptor. Researchers have established that the DOP-3 signaling pathway is necessary for the induction of paralysis, and that the *dop-3* knockout mutant *vs106* fails to undergo paralysis when treated with exogenous dopamine [23]. We confirmed this finding, as 50 mM dopamine failed to induce paralysis in *dop-3* mutants (Figures 2A and 2B). Similarly, when treated with either CBD or CBDV at concentrations ranging from 0.3 μM to 30 μM, *dop-3* mutants exhibited normal swimming behavior (Figures 2A and 2B).

3.3. Phytocannabinoid-induced paralysis in cat-2 mutants

The *cat-2* gene encodes tyrosine hydroxylase, which catalyzes the rate-limiting step in the dopamine biosynthetic pathway. *Cat-2* mutants cannot produce endogenous DA [14]. Paralysis was not observed in *cat-2* mutants treated with K medium alone or vehicle.

Treatment with 50 mM dopamine resulted in the paralysis of 60.9% ± 6.7% of the *cat-2* mutants. Similarly, *cat-2* mutants exposed to either CBD or CBDV showed a dose-dependent increase in paralysis, compared to vehicle. Specifically, CBD induced paralysis in 24.0% ± 3.7% of *cat-2* mutants, while CBDV elicited paralysis in 32,7% ± 4.6% of animals (Figures 3A and 3B).

3.4. Paralysis of wild type nematodes induced by phytocannabinoid and dopamine co-treatment

The effects of phytocannabinoid and dopamine co-treatment on wild type animals were analyzed. Compared to dopamine treatment alone, the fraction of paralyzed animals was significantly less in wild-type nematodes co-treated with dopamine and CBD (30.3% ± 5.8%) or dopamine and CBDV (36.3% ± 8.2%) (Figures 4A and 4B). Interestingly, there was no significant difference between the fraction of paralyzed animals following co-treatment compared to phytocannabinoid treatment alone, suggesting that phytocannabinoids are occupying the same receptors as dopamine, so that the latter could not exert its maximum effects.

4. Discussion

4.1. Phytocannabinoids as paralysis inducers via DOP-3

The dopaminergic system, consisting of endogenous dopamine, D1-like and D2-like dopamine receptors, and pre-synaptic dopamine transporters, is implicated in a broad range of neuropathologies and has consequently become an attractive drug target [3]. This neurotransmitter system is highly conserved in *C. elegans*, with homologs and orthologs for many of the mammalian genes responsible for dopamine synthesis, vesicular loading, dopamine reuptake and dopamine signaling[13].

In this study, we first used wild type nematodes to investigate the effects of two non-psychoactive phytocannabinoids on swimming behavior. Our results showed that both CBD and CBDV induce paralysis in wild type animals at concentrations in the micromolar range, suggesting that these phytocannabinoids may be acting on the dopamine pathway. We must note that we found that 50 mM dopamine induced paralysis in ~65% of wild type nematodes, while other groups have observed ~100% [23, 24]. This difference may be due to experimental conditions as dopamine was given on the agar plate in the previous studies [23, 24].

A lower concentration of either phytocannabinoids (30 μM), compared to 50 mM of dopamine, was needed to induce paralysis. This could suggest that these phytocannabinoids are more potent than dopamine. Alternatively, the requirement of a higher dopamine concentration may be associated with the difference in lipid solubility: hydrophobic phytocannabinoids versus polar, hydrophilic dopamine. The phytocannabinoids may more easily pass through the nematode cuticle to achieve their effects. In the current study, 30 μM was the highest concentration that could be achieved due to limited solubility of these phytocannabinoids. At this concentration, CBD- and CBDV-induced paralysis was at lower levels compared with those induced by dopamine. This could suggest that these

phytocannabinoids are less efficacious than dopamine and act as partial agonists on dopamine receptors.

To investigate the potential mechanism by which CBD and CBDV exerting their effects, two knock-out strains of *C. elegans* with loss-of-function mutations in the genes involved in the dopamine system, *dop-3* and *cat-2*, were examined.

The nematode DOP-3 receptor shares structural similarity to the mammalian D2-like dopamine receptors [7]. DOP-3 is expressed in the ventral cord motor neurons, which innervate the body wall muscles to directly control locomotion [7]. Specifically, high expression of DOP-3 is found in the GABAergic motor neurons that inhibit contractions; with only moderate expression in the cholinergic motor neurons that stimulate muscle contraction (overlapping co-expression of DOP-1 in cholinergic neurons). The *dop-3* (*vs106*) mutant contains deletions in sequences that encode the conserved transmembrane domains. As a results *dop-3* mutant has a loss of Swimming-induced paralysis phenotype [14]. In the current study, CBD and CBDV failed to induce paralysis in the *dop-3* mutant, which supports our hypothesis that CBD and CBDV act through the DOP-3 signaling pathway. The absence of paralysis in *dop-3* mutants and presence in N2 wild type animals suggest that CBD and CBDV act via the DOP-3 receptor to promote paralysis.

The *C. elegans* tyrosine hydroxylase ortholog CAT-2 mediates the rate-limiting step in dopamine biosynthesis, catalyzing the conversion of tyrosine to L-dopa [7]. The *cat-2* mutant (*e1112*) is deficient in dopamine, and as a result, does not demonstrate paralysis when immersed in solution under normal conditions. Instead, these animals can only undergo paralysis in the presence of exogenous dopamine or another DOP-3 agonist [7]. As expected, the *cat-2* mutants exhibited the paralysis phenotype when treated with dopamine. More importantly, treatment with CBD or CBDV induced paralysis in *cat-2* mutants, further confirming the agonist activity of CBD and CBDV on the dopamine pathway, presumably through dopamine receptor DOP-3. However, similar to the situation in N2 wild type, the levels of paralysis induced by these two phytocannabinoids in the *cat-2* mutant are only about 40-50% of that dopamine, suggesting again that these phytocannabinoids are partial agonists on DOP-3.

Our results suggest that CBD and CBDV may be acting as a partial agonist at DOP-3. We were then interested in whether these phytocannabinoids can block the effects of dopamine, because a partial agonist should compete and block the effects of a full agonist. To test this, we treated the wild type nematodes with the concentrations of dopamine and either CBD or CBDV that elicited the greatest paralysis effects. Co-administration of dopamine with either CBD or CBDV had reduced effects for dopamine to induce paralysis, and co-administration had similar effects on parylysis to either CBD or CBDV alone. These data further support the conclusion that phytocannabinoids CBD and CBDV act as partial agonists on dopamine receptor DOP-3.

4.2. Physiological significance and implications

Growing evidence supports the use of phytocannabinoids for the treatment of various neurological and psychiatric disorders, including Alzheimer's disease [25, 26], Parkinson's

disease [25, 26], neuropathic pain [27], epilepsy [28], anxiety [29], and schizophrenia [5, 30–32]. Interestingly, many of these pathologies are characterized by aberrant dopaminergic signaling.

It has been suggested that cannabinoids modulate mesocorticolimbic dopaminergic signaling [5]. Previously, ligand binding experiments *in vitro* has shown that CBD maybe a partial agonist at dopamine D2High receptors in rat striatal tissues[6]. Notably, this phytocannabinoid behaved similarly to the antipsychotic drug aripiprazole, an established dopamine partial agonist, suggesting a potential therapeutic use of CBD in the treatment of schizophrenia, where aberrant dopamine signaling is a hallmark [6]. The current study provides *in vivo* data, for the first time, to support the concept that the phytocannabinoids CBD and CBDV act as partial agonists on dopamine D2-like receptors. The dopamine hypothesis for schizophrenia posits that striatal hyper-dopaminergia causes the associated psychopathological symptoms. Urs et al. (2017) have further shown that it may be beneficial to downregulate overactive dopaminergic pathways in striatal tissues, while upregulating underactive dopaminergic pathways in cortical tissues [36]. Our finding has important implications for the mechanisms of actions of these phytocannabinoids for treating neurodegenerative and neuropsychiatric disorders, e.g. schizophrenia. One can imagine that a partial dopamine agonist such as CBD, CBDV as well as aripiprazole might be able to achieve this goal by blocking the D2 receptors where they are overreactive and activating the D2 receptor where they are underactive.

To our knowledge, this is the first report of CBD and CBDV as functional partial agonists for dopamine D2-like receptors *in vivo. C elegans* are simple but valuable models for studying molecular targets of pharmaceuticals. Now that a role of dopamine signaling has been established in nematodes for phytocannabinoids, further studies are warranted to extend the *in vivo* studies to the mammals, such as using mice with dopamine receptors knocked out, to explore the potential therapeutic effects and elucidate the detailed mechanisms of actions of phytocannabinoids on neurodegenerative and neuropsychiatric disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

CBD	cannabidiol
CBDV	cannabidivarin

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Highlights

- *C. elegans* were used to analyze the actions of cannabidiol and cannabidivarin
- These phytocannabinoids exhibited partial efficacy of dopamine in causing paralysis
- Knockout D2-like receptor, but not dopamine making enzyme, abrogated the effects
- Dopamine D2-like receptor is involved in the in vivo actions of phytocannabinoids
- This is a significant step toward understanding the mechanisms of phytocannabinoids

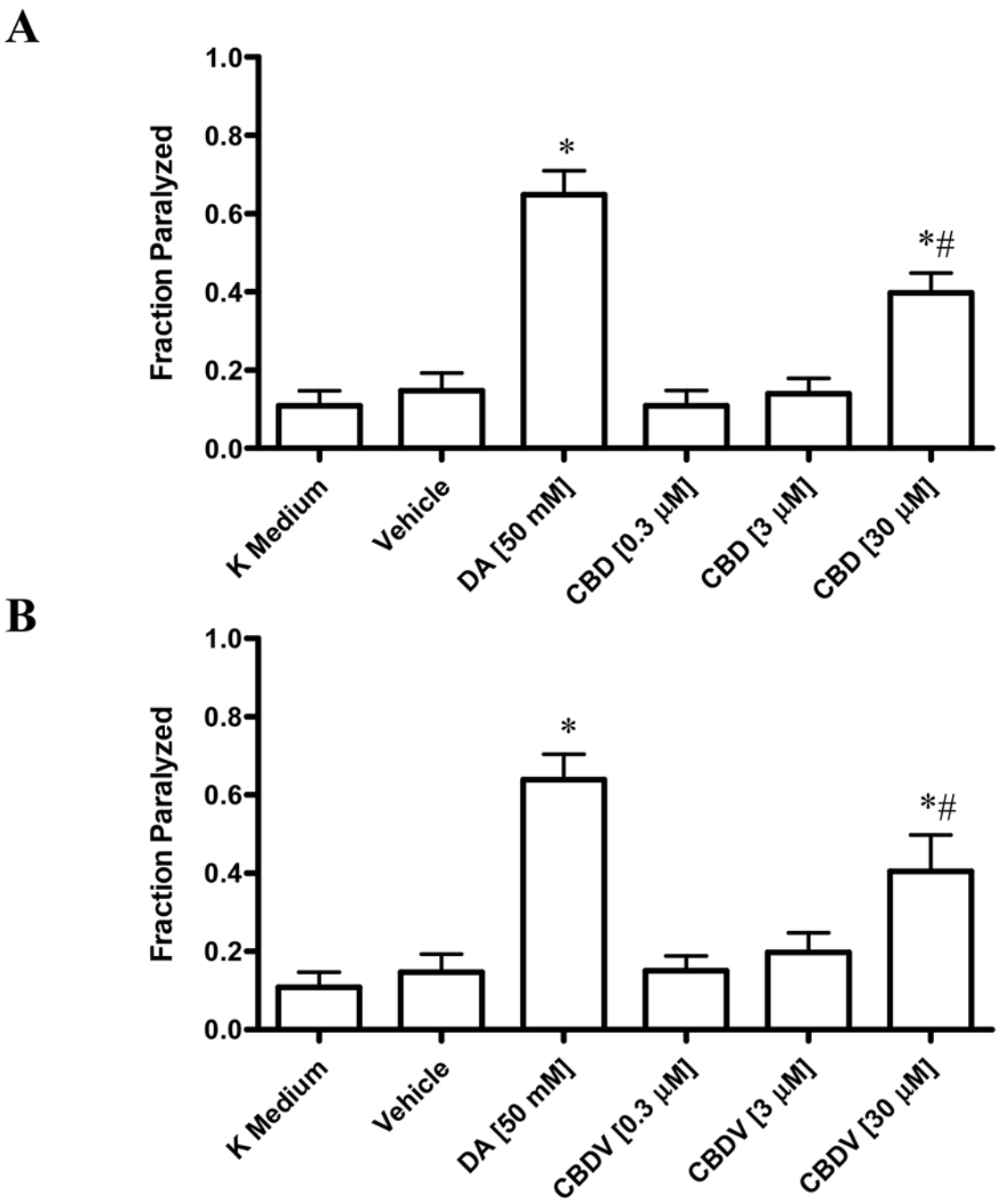

Figure 1. Phytocannabinoid-induced paralysis in N2 wild type nematodes.

Extent of paralysis induced by CBD (Fig. 1A) and CBDV (Fig. 1B) in N2 wild type nematodes. Data are expressed as mean ± standard deviation. Data were analyzed using one-way ANOVA followed by Dunnett's post hoc test. *p*-values of <0.05 were considered significant. * indicates statistically significant difference compared to vehicle. # indicates statistically significant difference compared to dopamine.

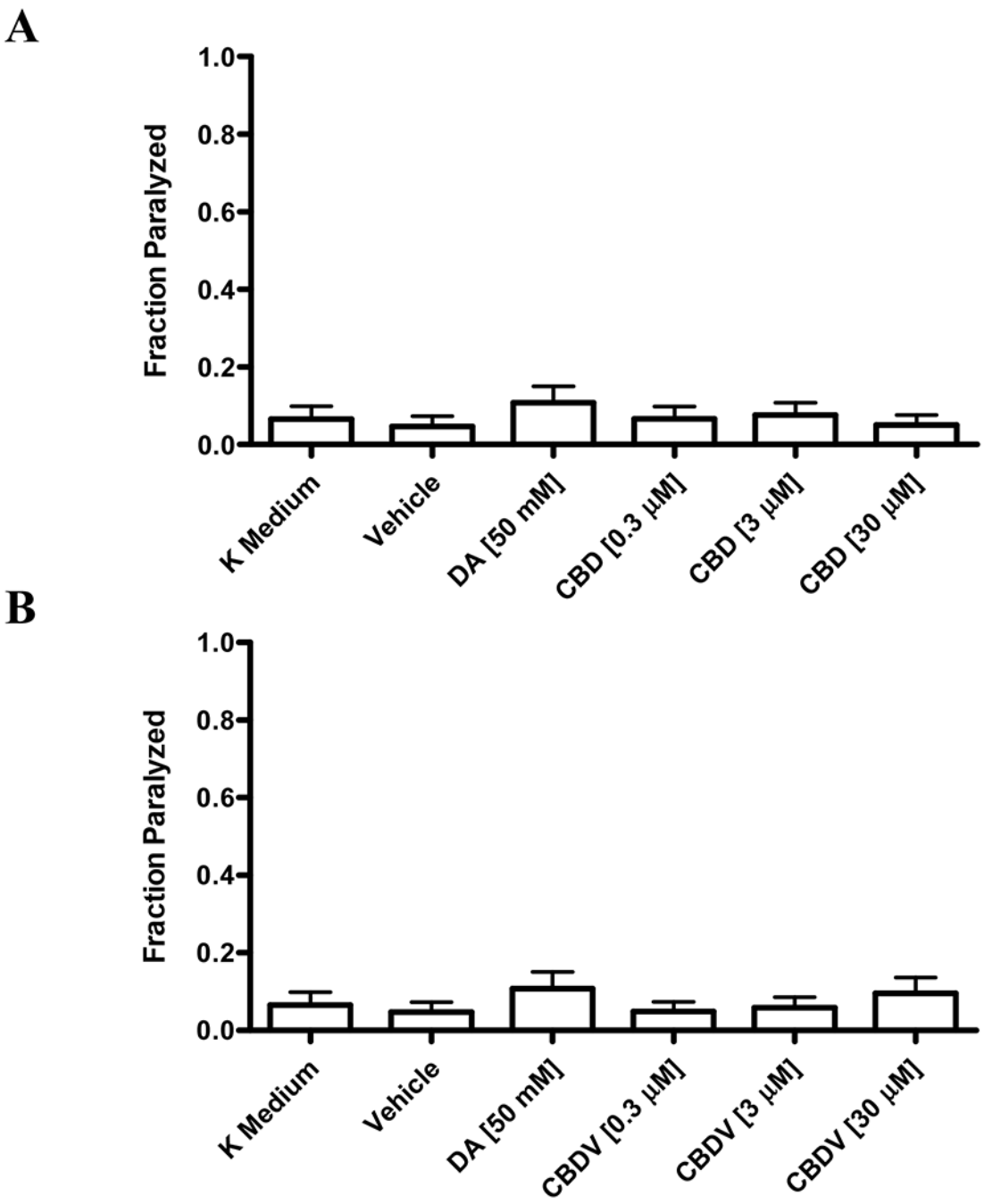

Figure 2. Lack of paralysis in phytocannabinoid-treated *dop-3* mutants.

CBD (Fig. 2A) and CBDV (Fig. 2B) fail to significantly induce paralysis in *dop-3* mutants lacking the D2-like DOP-3 receptor ($p > 0.05$). Data were analyzed using one-way ANOVA followed by Dunnett's post hoc test.

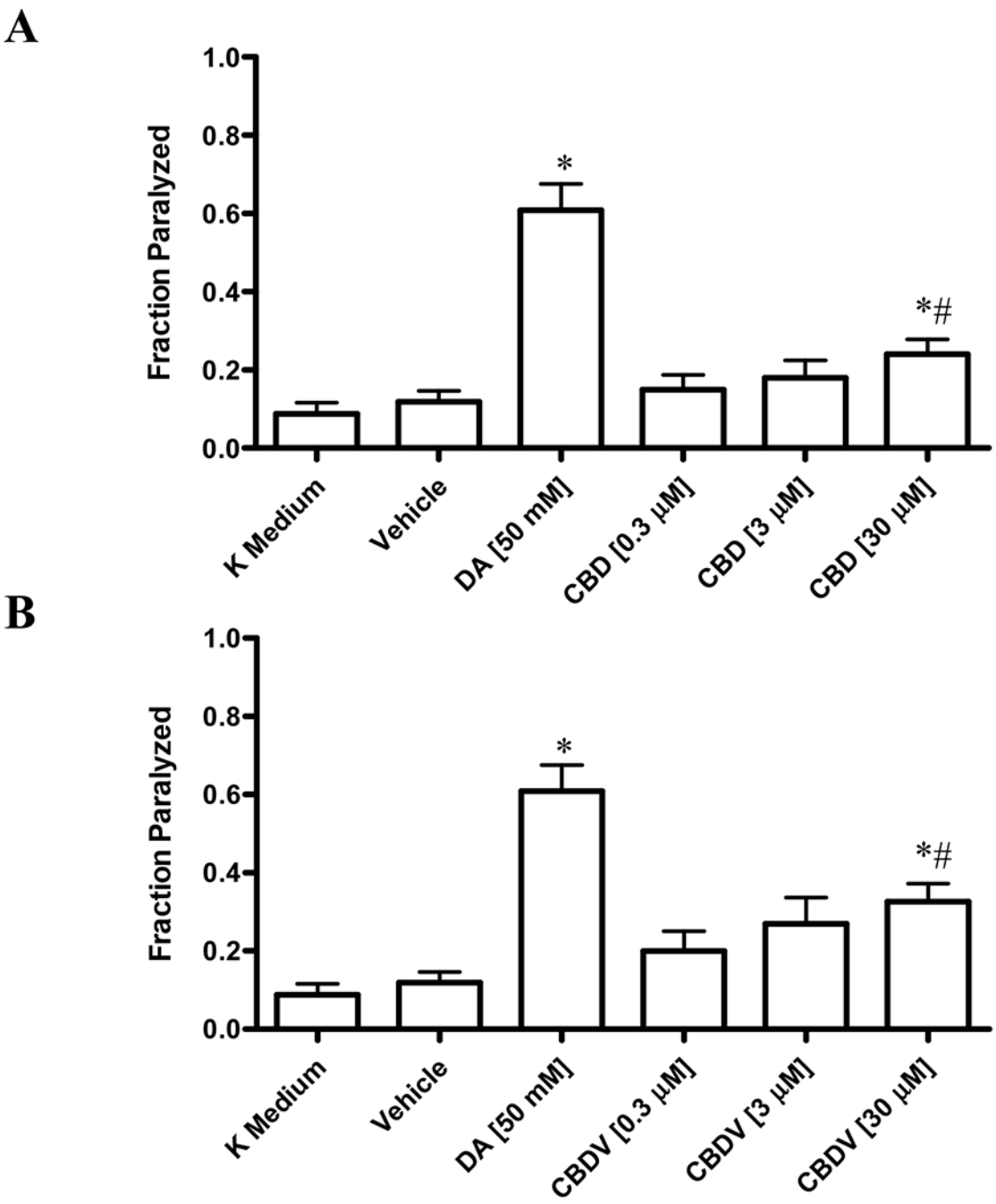

Figure 3. Phytocannabinoids-induced paralysis in *cat-2* mutants.

Extent of paralysis induced by CBD (Fig. 3A) and CBDV (Fig. 3B) in *cat-2* mutants. Data are expressed as mean ± standard deviation. Data were analyzed using one-way ANOVA followed by Dunnett's post hoc test. *p*-values of <0.05 were considered significant. * indicates statistically significant difference compared to vehicle. # indicates statistically significant difference compared to dopamine.

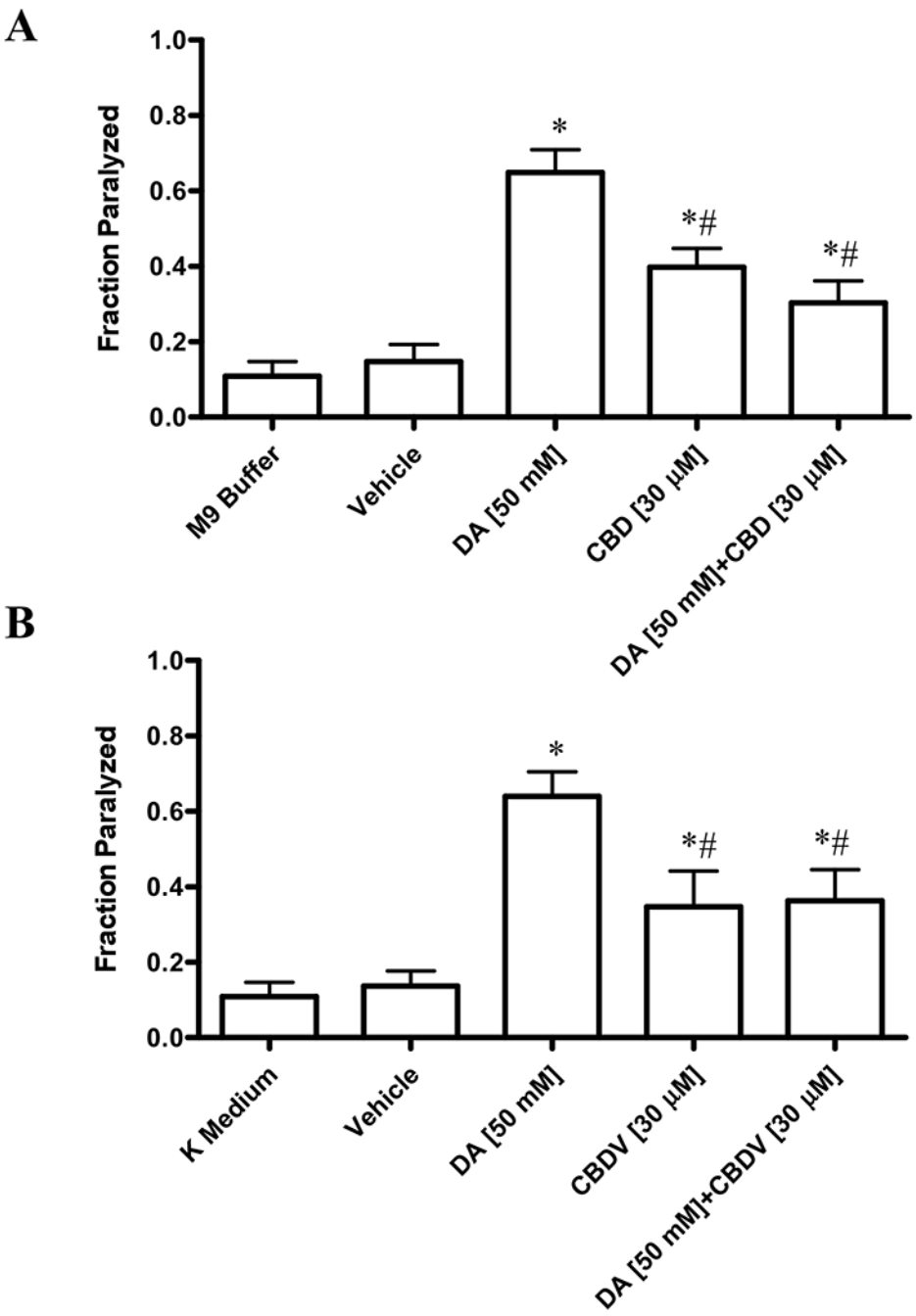

Figure 4. Co-treatment of phytocannabinoids and dopamine induced paralysis in N2 wild type nematodes.

Co-treatment of dopamine with 30 μM of CBD (Fig. 4A) or CBDV (Fig. 4B) induces lower levels paralysis compared to dopamine treatment alone. Data are expressed as mean ± standard deviation. Data were analyzed using one-way ANOVA followed by Dunnett's post hoc test. *p*-values of <0.05 were considered significant. * indicates statistically significant difference compared to vehicle. # indicates statistically significant difference compared to dopamine.